# METHOD FOR CONTROLLING ELECTRODEPOSITION OF AN ENTITY AND DEVICES INCORPORATING THE IMMOBILIZED ENTITY

## Background of the Invention

1. Field of the Invention

The present invention relates to a method and apparatus for controlling electrodeposition of an entity, such as a biomolecule, in which the entity is provided in the vicinity of a pair of electrodes in superposed relationship and a potential is applied across the electrodes sufficient to cause migration of the biomolecule component to one of the electrodes and cause deposition of a monolayer of the entity on the electrode. The invention further relates to methods of using the immobilized entity and to devices incorporating the immobilized entity.

2. Related Art

Conventional methods have been disclosed for immobilizing proteins on a substrate using chemical moieties. U.S. Patent No. 6,475,809 describes protein arrays for high throughpart screening in which a plurality of different members are immobilized on a surface of a substrate. A monolayer is provided on the surface of the substrate. The proteins are immobilized on the monolayer. The monolayer is formed of a variety of chemical moieties including alkysiloxane monolayers, alklthiol/dialkyldisulfide monolayers and an alkyl monolayer on an oxide free silicone substrate.

U.S. Patent No. 4,294,677 describes a method for electrodepositing a protein by electrophoresis onto an ion-exchange membrane from a liquid in which the protein is dissolved or is dispersed in suspension. The ion exchange membrane may comprise chemically resilient highly bridged polymeric skeletons on which many anion and cation exchange groups such as sulfonate group, carboxylate group, phenol group and ammonium group are attached as substituents.

Other conventional methods for electrodepositing a protein without using a chemical moiety have been described.

U.S. Patent No. 5,166,063 describes a method for immobilizing molecules on a conductive substrate to produce a biosensor. A biosensor electrode and a counter electrode are immersed in a container of a solution of at least one species of biomolecule. A potential difference of less than 1 volt is created between the electrodes. This patent has the drawback that

because of the relatively large volume used in the system it is difficult to control the amount of the biomolecule that is accumulated on the biosensor electrode.

It is desirable to provide a method and system for controlling electrodeposition of an entity.

Summary of the Invention

The present invention relates to a method and system for controlling electrodeposition of a deposition entity in which a solution or suspension of the deposition entity is provided between a pair of superposed electrodes at a predetermined concentration. A potential is applied to the electrodes sufficient to cause migration of the deposition entity to one of the electrodes and deposition of a controlled thickness of the deposition entity. The distance between the electrodes and voltage applied can be controlled to provide migration of the deposition entity. The method and system provide controlled immobilization of deposition entities such as proteins, enzymes, light harvesting complexes, DNA, RNA, PNA onto a substrate without loss of function. In one embodiment, the system can be used on a nanoscale. Additionally, devices can be formed by the method of the present invention. The invention will be more fully described by reference to the following drawings.

Brief Description of the Drawings

Fig. 1 is a schematic cross sectional view of a system for controlling electrodeposition of a deposition entity in accordance with the teachings of the present invention.

Fig. 2 is a top view of a retainer housing of the system shown in Fig. 1 in combination with an electrode.

Fig. 3A is a graph of absorption spectra of a deposited film of a deposition entity produced by a device according to the present invention.

Fig. 3B is a SEM micrograph of the film shown in Fig. 3A.

Detailed Description

Reference will now be made in greater detail to a preferred embodiment of the invention, an example of which is illustrated in the accompanying drawings. Wherever possible, the same reference numerals will be used throughout the drawings and the description to refer to the same or like parts.

Fig. 1 is a schematic diagram of a system for controlling electrodeposition of a deposition entity 10 in accordance with the teachings of the present invention. System 10 includes electrode 12 and electrode 14. Electrode 12 and electrode 14 are in a superposed relation.

Electrodes 12 and 14 can be formed of metals or "metal substitutes." The term "metal" is used to embrace both materials composed of an elementally pure metal, such as Ag or Mg, and also metal alloys which are materials composed of two or more elementally pure metals, e.g., Mg and Ag together, denoted Mg:Ag. The term "metal substitute" refers to a material that is not a metal within the normal definition, but which has the metal-like properties that are desired in certain appropriate applications. Suitable metal substitutes which can be used for electrodes 12 and 14 include doped wide bandgap semiconductors, for example, transparent conducting oxides such as indium tin oxide (ITO), gallium indium tin oxide (GITO), and zinc indium tin oxide (ZITO). Other suitable materials for electrodes 12 and 14 are polymeric metals such as poly-ehtylene-dioxythiophene (PEDOT) doped with poly-styrenesulfonate (PSS).

One or more of electrode 12 and electrode 14 can be transparent. As used herein, a layer of material is said to be "transparent" when the layer or layers permit at least 50% of the ambient electromagnetic radiation in relevant wavelengths to be transmitted through the layer or layers. Similarly, layers which permit some but less than 50% transmission of ambient electromagnetic radiation in relevant wavelengths are said to be "semi-transparent". In particular, ITO is a highly doped degenerate $n^+$ semiconductor with an optic bandgap of approximately 3.2 eV rendering it transparent to wavelengths greater than approximately 3900 Å. Another suitable metal substitute material is the transparent conductive polymer polyanaline (PANI) and its chemical relatives.

Metal substitutes can be further selected from a wide range of non-metallic materials, wherein the term "non-metallic" is meant to embrace a wide range of materials provided that the material is free of metal in its chemically uncombined form. When a metal is present in its chemically uncombined for, either alone or in combination with one or more other metals as an alloy, the metal may alternatively be referred to as being present in its metallic form or as being a "free metal". Thus, the metal substitute electrodes of the present invention may sometimes be referred to as "metal-free" wherein the term "metal-free" is expressly meant to embrace a material free of metal in its chemically uncombined form. Free metals typically have a form of metallic bonding that may be thought of as a type of chemical bonding that results form a sea of valence electrons which are free to move in an electronic conduction band throughout the metal

lattice. While metal substitutes may contain metal constituents they are "non-metallic" on several bases. The are not pure free-metals nor are they alloys of free-metals. When metals are present in their metallic form, the electronic conduction band tends to provide, among other metallic properties, a high electrical conductivity as well as a high reflectivity for optical radiation.

Electrode 12 can be attached to substrate 15 and electrode 14 can be attached to substrate 16. For example, electrode 12 and electrode 14 can be deposited as a film on respective substrate 15 and substrate 16 with known metal and non-metal deposition techniques such as electron beam evaporation and the like.

Substrates 15 and 16 can be either organic or inorganic, biological or non-biological, or any combination of these materials. In one embodiment, the substrate is transparent or translucent. Substrates 15 and 16 can be flat, firm or semi-firm. Suitable materials for substrates 15 and 16 include silicon, silica, quartz, glass, controlled pore glass, carbon, alumina, titanium dioxide, germanium, silicon nitride, zeolites, and gallium arsenide. Metals such as gold, platinum, aluminum copper, titanium, and their alloys are also options for the substrates. In addition, many ceramics and polymers can also be used as substrates. Polymers which can be used as substrates include, but are not limited to, the following: polystyrene; poly(tetra)fluorethylene; (poly)vinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinlyethylene; polyethyleneimide; poly(etherether) ketone; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyalkenesulfone (PAS); polyhydroxyethylmethacrylate; polydimethylsiloxane; polyacrylamide; polyimide; co-block-polymers; and Eupergit®, Photoresists, polymerized Langmuir-Blodgett films, and LIGA structures can also serve as substrates in the present invention.

Power supply 18 having positive lead 19 connected to electrode 12 and negative lead 20 connected to electrode 14 is provided to supply substantially constant current flow between electrode 12 and electrode 14. The direction of current flow can be reversed if desired by switching the connections of lead 19 and lead 20 to power supply 18 to make lead 19 negatively charged and lead 20 positively charged.

Distance $D_1$ between electrode 12 and electrode 14 can be in the range of about 10nm to about 5.0mm. In one embodiment, the distance $D_1$ and size of electrode 12 and electrode 14 are selected to be useful in nanoscale devices. Deposition on nanoscale electrodes can occur

provided the remaining area of the substrate is insulated. A suitable distance $D_1$ is about 1.0mm. The voltage applied to electrode 12 and electrode 14 is dependent on the distance $D_1$. For example, the voltage applied can be in the range of about 1 V/cm to about 1,000 V/cm. A suitable voltage range of about 10 V/cm to about 200 V/cm can be used with a distance between electrode 12 and electrode 14 of about 1mm.

A solution or suspension of deposition entity 22 is provided between electrodes 12 and 14. The voltage is continuously applied for a predetermined time to effect migration of deposition entity 22 toward electrode 12 or 14 to provide deposition of a film of deposition entity 22 on electrode 12 or electrode 14. For example, voltage can be continuously applied for about 5 minutes to about 48 hours. The voltages applied are based on the desired thickness of a film of deposition entity 22, and on the concentration of the solution from which deposition entity 22 is electrodeposited. It has been found desirable to use the smallest distance between electrodes 12 and 14 in order to decrease the voltage to provide needed migration of deposition entity 22.

The concentration of the deposition entity in solution or suspension of deposition entity 22 and the volume of the solution is selected to control the thickness of a film of deposition entity 22 that is deposited on electrode 12 or electrode 14 upon continuous application of a predetermined voltage. For example, the concentration of the deposition entity in solution or suspension of deposition entity 22 can be selected to form a monolayer on electrode 12 or electrode 14. In one embodiment of the present invention, 100% of the deposition entity can be deposited on electrode 12 or electrode 14 using a concentration of the deposition entity in the range of about 10μg/ml to about 1mg/ml , a volume of about $1mm^3$ to about $100mm^3$ with a voltage in the range of about 10 V/cm to about 200 V/cm resulting in a film of a monolayer having a thickness of about 5nm to about 10nm. It will be appreciated that thicker films can be deposited by varying the concentration of deposition entity 22 in solution or suspension and the volume of the solution.

Retainer housing 24 can be used to retain solution or suspension of deposition entity 22 between electrodes 12 and electrodes 14. Retainer housing 24 is positioned adjacent electrode 12 and electrode 14. As shown in Fig. 2, retainer housing 24 can have open ends, such as an O-ring. Alternatively, retainer housing 24 can have various shapes. Retainer housing 24 can have a size selected to provide a predetermined volume of solution or suspension of

deposition entity 22. For example, retainer housing 24 can have a size to provide a volume of about $1mm^3$ to about $100mm^3$.

In one embodiment, retainer housing 24 can be placed on one electrode for example, electrode 14. Thereafter, a solution or suspension of deposition entity 22 is received in retainer housing 24 and contacts electrode 14. The volume of the solution or suspension of deposition entity 22 fills retainer housing 24. The other electrode for example, electrode 12 is placed on top of retainer housing 24 for retaining deposition entity 22 between electrode 12 and electrode 14. For example, a substrate can be used with retainer housing 24 300mm silicon wafer on the order of $10^5mm^3$ to cover the whole substrate with about a 1mm thick deposition cell.

Migration of the deposition entity occurs towards the electrode 12 or 14 charged in the opposite sense to the charge of the deposition entity in solution or suspension of deposition entity 22. Upon migration of deposition entity 22 to electrode 12 or electrode 14, deposition entity 22 can be attached to electrode 12 or 14 largely due to van der Waals interactions between the deposition entity and electrode 12 or electrode 14.

The deposition entity is suitable for deposition on electrodes 12 or 14. Suitable deposition entities include but are not limited to the following classes of naturally occurring or artificially synthesized molecules or molecular grouping that can exist as components of biological systems: proteins including simple proteins and complex proteins containing other organic compounds, such as for example apoproteins, glycoproteins, peptides, oligopeptides, lipoproteins, ovo-proteins, lacto-proteins, serum-proteins, myo-proteins, seed-proteins, scleroproteins, chromoproteins, phosphoproteins and nucleo-proteins. Other suitable deposition entities include antigens and antibodies thereto, antibody fragments, haptens and antibodies thereto, receptors and other membrane proteins, protein analogs in which at least one non-peptide linkage replaces a peptide linkage, enzymes and enzyme precursors, coenzymes, enzyme inhibitors, amino acids and their derivatives, hormones, lipids, phospholipids, glycolipids, liposomes, nucleotides, oligonucleotides, polynucleotides, and their art-recognized and biologically functional analogs and derivatives including, for example: methylated polynucleotides and nucleotide analogs having phosphorothioate linkages; plasmids, cosmids, artificial chromosomes, other nucleic acid vectors; antisense polynucleotides including those substantially complementary to at least one endogenous nucleic acid or those having sequences with a sense opposed to at least portions of selected viral or retroviral genomes, viruses, bacteria

phages, antisense and any other biologically active molecule, synthetic composite, macromolecules or synthetic polymers. Suitable deposition entities 22 also include deoxyribonucleic acids (DNA), ribonucleic acids (RNA) and peptide nucleic acids (PNA).

Deposition entity 22 can include a light harvesting complex. The term "Light Harvesting Complex" (LHC) as used herein refers to photosynthetic complexes, e.g., PSI (Photosystem I, from spinach, for example), PS2 (Photosystem II), LH1 (Light Harvesting complex 1) and/or LH2 (Light Harvesting complex 2, from purple bacteria). Fromme, P., *et al.*, *Biochim. Biophys. Acta* 1365, 175 (1998); Lee, I., *et al.*, *Phys. Rev. Lett.* 79, 3294 (1997); Schubert, W.D., *et al.*, *J. Mol. Biol.* 272, 741-768 (1997). These complexes are available commercially, for example, from PROTEIN LABS Inc., 1425 Russ Blvd., Suite T-107C, San Diego, CA 92101. Any of the preceding deposition entities having weak or non-existent polarity or induceable polarity under the conditions prevailing in system 10 can be covalently linked to an appropriate charged carrier to form a charged complex that can be deposited on the electrodes 12 or 14.

Members of the preceding classes of deposition entities and any combination of specific members thereof can be placed in solution or in suspension as colloidal particles in liquid using art recognized techniques that depend on the composition of the liquid. The solution or suspension of deposition entity 22 can be an aqueous solution, such as physiological saline, capable of conducting a substantial electrical current. The solution or suspension can have a desired pH at a physiological level. The direction, rate of migration, and rate of deposition of the deposition entity originally in solution or suspension of deposition entity 22 onto electrodes 12 and 14 can be controlled with great sensitivity by appropriately adjusting the pH of the solution. This control is based upon use of conventional electrophoretic techniques applicable to permanently charged moieties that give the deposition entity a net charge in the solution depending on the pH of the solution. The pH at which the deposition entity has zero net negative charge, and thus will not migrate under the influence of an electric field, is defined as its isoelectric point. At pH values greater than the isoelectric point, the molecule has a net negative charge; conversely at pH values less than the isoelectric point, the molecule has a net positive charge. Accordingly, in system 10 shown in Fig. 1, the pH of the solution or suspension of deposition entity 22 is adjusted to greater than or less than the isoelectric point of the deposition entity to be deposited on electrodes 12 or 14. This adjustment can be accomplished using known

acids or alkaline agents as desired. Other additives, such as non-ionic surfactants and anti-foaming agents or detergents can also be added to the solution as desired.

Immobilized deposition entities produced according to the method and system of the invention can be used in a wide variety of molecular detection systems, including amperometric electrochemical biosensors, calorimetric, acoustic, potentiometric, optical, and ISFET based biosensors.

Immobilized entities such as proteins, enzymes, antibodies, or glycoproteins such as lectins can be used in biosensors that detect the presence or concentration of selected physiological molecules as a result of the interaction of the physiological ligand with the immobilized biomolecules.

Immobilized entities can be used in any device in which the immobilized entity is essential to operation of the device. Suitable devices include solid state devices, memory devices and photo voltaic devices.

Fig. 3A illustrates absorption spectra of a film of LH2 deposited onto an electrode. A pair of electrodes had about 1mm electrode separation. A voltage of about 50 volts was applied for 24 hours at room temperature. The absorption spectra shows peaks at 800nm and 850nm are clearly visible indicating the complexes are intact (the absorption of unassociated pigment molecules would be blue shifted).

Fig. 3B is a SEM micrograph of the resulting film. The 10nm - 15nm sized features are the complexes of interest.

It is to be understood that the above-described embodiments are illustrative of only a few of the many possible specific embodiments which can represent applications of the principles of the invention. Numerous and varied other arrangements can be readily devised in accordance with these principles by those skilled in the art without departing from the spirit and scope of the invention.